Sequential exposure to biological and chemical contaminants: a new *in vitro* model on human respiratory cells

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Background and Aims:
Humans are permanently exposed to numerous pollutants of different types. The effects of the association between chemical and biological compounds on human respiratory health, and especially asthma, are still unclear. In order to clarify these effects, toxicological evidence is needed in addition to epidemiological observations.
Our aim was to develop an *in vitro* model of exposure using two types of pollutants. The individual or combined *in vitro* effects of a chemical pollutant and a biological contaminant, known to be found in domestic environment and deleterious to respiratory health, were assessed *in vitro* on epithelial respiratory cells using a unique exposure device and stringent conditions of exposure.

Methods:
Human alveolar epithelial cells (A549) were sequentially exposed during 30 min, each at the air-liquid interface in an Vitrocell exposure module to FA at an environmental level (50µg/m^3) then to viable spores of Asp (8.10^8 spores/m^3, sampled at the inflow level). Controls comprised sequential exposures to each agent alone or to ambient filtered air. After 10h post-incubation, cellular viability (XTT and LDH assays) was assessed. Biomarkers of local inflammation, IL-8 and MCP-1 were assayed by ELISA in the medium removed from apical face of cells, and their mRNA expression was quantified by RT-PCR.

Results:
Using this protocol, sequential exposure to air, FA or Asp did not impact cellular viability. Single exposure to FA or Asp and sequential exposure to FA then air did not induce significant changes of production or expression of inflammatory cytokines. However, sequential exposure to air then Asp tended to induce IL-8 production and expression. When combined, FA followed by Asp exposure increased IL-8 production and MCP-1 expression.

Conclusions:
These results show that a combined exposure to different types of environmental pollutants can be modelled in vitro.